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CERTAIN RESULTS OF PHYSIOLOGICAL AND ECOLOGICAL
INVESTIGATIONS OF THE CHLORELLA CULTURE AS A LINK IN A
CLOSED ECOLOGICAL SYSTEM

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ABSTRACT

Unicellular algae including chlorella, represent
one of the leading components of biological life-support
systems on spacecrafts. The material balance of that
portion of the photosynthesis link based on utilizing
unicellular algae is discussed.

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K. E. Tsiolkovskiy (Ref. 9) proposed that the principle of the /1*
biological cycle in substances be employed in providing life-supporting
systems for the crew on spacecrafts. This represented a natural outcome
of his work on the theoretical basis of interplanetary flights.

It is interesting to note that the first attempts to provide an
experimental approach to this problem were already undertaken in 1915-
1919 by engineer A. F. Tsander, one of the founders of rocket construc-
tion in the USSR. In experiments on the cultivation of vegetables and
artificial substitutes for soil (charcoal), he employed food
products for the human being to feed them (Ref. 8). These were apparently
the first experimental-ecological studies directly arising from the needs

* Note: Numbers in the margin indicate pagination in the original foreign
text.

of cosmonauts.

At the present time the idea of creating a closed cycle of substances has become the subject of not only experimental studies, but also structural undertakings carried out by many laboratories in several countries.

Unicellular algae represent one of the first proposed components of biological life-support systems on spacecrafts. In particular, this includes chlorella which is now regarded as one of the most probable elements of the photosynthetic link in a closed ecological system.

The present report is devoted to studying primarily the material /2 balance of that portion of the photosynthesis link which may be based on utilizing unicellular algae. In examining this problem, we must not overlook certain general aspects of ecological system modeling, because they determine the main paths which are followed in the development of individual functional links. In contrast to animate nature, which represents a complete system in all stages of its development and never arises due to synthesis from previously-existing elements, we are forced to follow this path of "single-act creation", creating a functionally complete system from the beginning elements which are adapted to performing their function within the framework of another, already-existing system - nature on the earth.

In view of the complexity of this problem which has no precedence, the choice of the general method by which it is solved is very important. It is clearly apparent that the method of direct synthesis by means of trial and error is unsuitable here, due to the extreme complexity and not always predictable extent of the direct and reverse connections between elements in the created system. In the given case, the only

suitable method is the systematic construction of the system as a whole and the elements corresponding to it, on the basis of preliminary calculations. In other words, a closed ecological system must be created from the very beginning as a complete system, with fully-determined aims and functional characteristics, which determine its composition, structure, and basic parameters of the component elements.

It follows from the statements given above that the construction of experimental models of closed ecological systems is based, to a considerable extent, on the study and quantitative description of those functional relationships between previously-selected objects which determine their position, needs, and external operation in a cyclical system of matter and energy - their so-called ecological niche.

We are thus discussing a study and description of the input and output characteristics of proposed elements in a future system. These characteristics represent the necessary condition for preliminary calculations and effective development of models for biological systems having any definite purpose.

We would like to point out the following basic characteristics, necessary for calculations and modeling, for the unicellular algae link, which is the subject of this report.

A. Input Parameters

- Energy: Optimum and extreme values of intensity, spectral composition; incoming regime, specific requirements.

- General Operational Conditions: Optimum and extreme values of temperature, acidity of the medium, dynamic conditions of cultivation - mechanical overloads, pressure, and their decrease.

- Material Requirements: Optimum and extreme concentrations /3
of carbonic acid and mineral elements, their specific requirements.

B. Output Parameters

- Energy: The portion of free energy which can be applied
in the system, and its relationship to decreasing and absorbed energy.

- Material Output: Specific productivity with respect to oxygen
and biomass, chemical composition and nutritive value of biomass, its
coefficient of effective utilization in the system.

- Secondary Processes: Contamination of the atmosphere with
foreign substances, accumulation of products arising from metabolism
and cell destruction in the feeding medium.

In addition, automatic control and regulation in the system
require a study of certain special problems encompassing the general
properties of the link in the information and control system: the in-
formative value of individual link parameters, frequency characteristics of
spontaneous and forced oscillatory processes, parameters of transitional
processes and transmission functions, etc.

This report includes several results derived from a study of
those characteristics of the material balance in the chlorella culture
which are necessary for determining its location, needs, and role in
gas-exchange systems or closed cyclical systems for substances. Several
data are also presented on the dynamic properties of the chlorella culture
as the object of control.

In the Soviet Union, a study of the unicellular algae culture for
practical utilization in air regeneration systems has been prepared by
researchers N. S. Gayevskaya, V. V. Pinevich, V. A. Chesnokov, N. N.

Verzilin et al (Ref. 1, 5, 10) and also V. Ye. Seminenko, M. G. Vladimirova, and A. A. Nichiporovich (Ref. 7).

A group of our co-workers in 1960-1961 (G. I. Meleshko, Ye. K. Lebedeva, T. B. Galkina, etc.) made the first attempt to provide for gas-exchange in animals and man due to chlorella photosynthesis in systems which were closed with respect to gas-exchange. At a later period we undertook a complex study of the chlorella culture as a possible link in a closed ecological system. The data from this study provide the basis for this report.

The first experiments providing for gas-exchange in animals made it possible to draw the conclusion that successful experimental modeling of ecological systems requires the most accurate possible knowledge of material-energy requirements, operational conditions, and material outputs of each element in the system in order to reproduce them in the model.

The direct utilization of data obtained from physiology for carrying out a stable and highly-productive biotechnological process in algae populations is rendered more difficult by the fact that the transforming effect of the population on the feeding medium is not taken into account in the general physiological characteristics. At the same time, as a 14 result of this influence the conditions in the surrounding medium do not always remain constant within the population. The greater the density of the population and its specific productivity, the more these conditions deviate from the original value at the boundaries of the territory which it occupies (biotope).

Due to this fact, intra-population gradients of the medium properties

develop - this is an important characteristic which distinguishes the population as a system from a simple multiplicity of individuals. Therefore, the organization of special experimental-ecological studies is necessary to provide a quantitative determination of the functional relationships for a given link in the ecological system. The object of these studies would be a sufficiently-productive population of an investigable type, with all the features of intra-population relationships for individual elements and the interrelationships for the population as a whole with the surrounding medium.

In order to obtain the requisite data, a group of co-workers under the leadership of G. I. Meleshko carried out complex studies on the material balance of chlorella cultivated in photosynthetic reactors having a high output with a short, closed air circuit.

One of the important characteristics of a photosynthetic link is the gas-exchange coefficient - the volumetric relationship of the carbonic acid required to the oxygen liberated; this relationship should correspond to the respiratory coefficient of the consuming links in the ecological system. This quantity was determined experimentally on the basis of directly measuring the carbonic acid and oxygen balance in a hermetic system. This quantity proved to be 0.79 ± 0.01 for a rather significant dispersion (standard deviation 0.066).

It should be noted that the value for the gas-exchange coefficient does not depend on the suspension density in the region between $3 \cdot 10^8$ and $1 \cdot 10^{10}$ cells per m^3 or on the photosynthesis intensity between 0.15 and 6.0 l CO_2 per hour from one liter of suspension. This level for the gas-exchange coefficient was obtained for chlorella cultivated in a

Tamiyya medium, where the nitrogen source was nitrate.

When the nitrogen source in the medium was urea, the gas-exchange coefficient increased to 0.995 ± 0.022 . When the algae were cultivated in liquid and solid human foodstuffs, which had been previously mineralized in an aerobic bacteria reactor, the gas-exchange coefficient approximated its level in nitrate media and amounted to 0.82. As can be seen from the data obtained, the gas-exchange coefficient for the chlorella culture in mineral and mineralized organic media lies within the customary limits for the respiratory coefficient of man.

The intensity of the photosynthesis, the gas balance, the mineral feeding of the algae, and their elementary and biochemical compositions were studied simultaneously in a special series of investigations. A 15 portion of these data are shown in Table 1, in which it can be seen that 1 l of carbonic acid is absorbed on the average, and 1.23 l of oxygen is liberated (at 20°), during the formation of 1 g of dry algae substance. The relationship which was found is retained within a rather wide range of the culture density, as well as the intensity of the photosynthesis, the acidity of the medium, and the exposure.

A fairly stable correlation between the studied indices of photosynthesis intensity makes each of them suitable both as a criterion for culture productivity, and as a source of control signals for the automatic regulation of basic growth conditions in the algae culture. For this purpose, in subsequent experiments G. I. Meleshko successfully used an automatic carbonic acid-analyzer with attitude control.

At the same time these experiments investigated the dynamics by

TABLE 1
RELATIONSHIP BETWEEN GROWTH OF BIOMASS, ABSORPTION OF
CARBONIC ACID, AND LIBERATION OF OXYGEN IN INTENSE
CHLORELLA CULTURE

(1) Плотность суспензии, г/л	(2) Прирост биомассы, г сухого веса	(3) Поглощение CO ₂ на 1 г сухого в-ва, л	(4) Выделение O ₂ на 1 г сухого в-ва, л
2,2	0,19	1,00	1,23
3,75	0,28	0,97	1,11
4,5	0,40	1,03	1,20
5,6	0,29	0,97	1,21
6,75	0,42	1,04	1,24
11,2	0,32	1,00	1,11
18,0	0,46	1,04	1,38
25,7	0,47	1,02	1,36
30,0	0,60	0,98	1,23
M ± m	—	1,04 ± 0,04	1,23 ± 0,03

(1) - Suspension Density, g/l; (2) - Biomass Growth, g of Dry Weight; (3) - CO₂ Absorption Per 1 g Dry Substance, l;
(4) - O₂ Liberation Per 1 g Dry Substance, l

which mineral nutritional elements were removed from the culture medium, the accumulation of these elements in the algae cells (Table 2), as well as the biochemical composition of the cell substance.

A comparison of the decrease in elements from the feeding medium with an accumulation in the biomass shows that a portion of them is again liberated in the culture medium, probably in the form of products derived from cell metabolism or destruction, which comprise a relatively small 16 amount for a high culture productivity.

The chlorella biomass obtained under these conditions is characterized by the molecular formula C_{6.6}H_{12.2}O_{3.5}N_{1.0} and contains: ash 7.3% dry weight, albumen 56.3%, fat 8.5%, carbohydrates including cellulose 35.2% organic substance.

TABLE 2
ACCUMULATION OF BASIC ELEMENTS IN ALGAE CELLS

Elements	Per mg/g Dry Substance	In Proportion to Decrease From Medium, %
Nitrogen	80.0	96.5
Phosphorus	17.4	98.8
Sulfur	11.2	-
Potassium	16.0	-
Magnesium	5.7	99.1
Iron	0.3	-
Carbon	500.0	92.4

Theoretically, the consumption of a substance having the formula shown above yields a quantity for the gas balance which closely coincides with that obtained experimentally.

As a result of the study, the authors obtained complex characteristics for an intense chlorella culture. These characteristics expressed a quantitative dependence between the gas balance in the culture, the consumption of mineral elements from the culture medium, their accumulation in the cells, and the production of biomass having a definite composition. These data provide a basis for the subsequent development of methods encompassing prolonged chlorella cultivation. One of the purposes of the investigation was to discover the combination of algae cultivation conditions which would provide for a rather high specific productivity of their suspension. This combination was found and realized in the film cultivation method, which made it possible under laboratory conditions to increase the culture productivity to 8-10 l oxygen with 1 l suspension per hour - i.e., to attain values which are commensurate with the potential possibilities of the chlorella culture (Ref. 5).

It was possible to achieve this level of productivity, because the optimum level of the basic cultivation conditions were revised in conformity with the specific features of a high-density population. In particular, the experiments of G. I. Meleshko and L. M. Krasotchenko revealed that the levels of carbonic acid saturating concentrations were higher than those indicated by data from present day laboratory practice. They amounted to (in air) 1.5-1.8 and 4.5-5.5% for a suspension density /7 of $0.5-1 \cdot 10^9$ and $4-5 \cdot 10^9$ kl/ml, respectively.

The accumulation of products which are biologically active in the culture medium is of practical importance for the cultivation of algae in suspensions having high density and high specific productivity. The interesting experiments of T. B. Galkina (Ref. 2) showed that the stimulating effect of the culture medium initially decreased as the suspension density increased, and then it changed into an inhibiting effect. The transition point is the "physiological growth" of this medium, during which approximately 500 million cells adhere to each millimeter of the medium, independently of the actual cultivation time.

When the experiment was formulated in just the opposite manner, the same medium was systematically diluted, which had a clearly-expressed inhibiting effect. It was thus found that the inhibiting effect decreases with dilution, and when the medium is further diluted it changes into a stimulating effect. These data clearly point to the common nature of the stimulating and inhibiting effect of some substances accumulated in the medium during prolonged cultivation of algae.

The content of certain gaseous impurities in the closed air circuit of the reactor (Ref. 4) was determined in several experiments in this

group of investigations. In addition to confirming already-established data on the liberation of carbon monoxide by the chlorella culture (Ref. 11, 13, 15, 16), the experiments showed that the specific accumulation of this gas is inversely proportional to the photosynthesis intensity in the culture (Table 3).

TABLE 3
ACCUMULATION OF CARBON MONOXIDE PER 1 g INCREASE IN THE
DRY CHLORELLA SUBSTANCE FOR DIFFERENT CULTURE
PRODUCTIVITY (TAMIYYA MEDIUM)

Dry Substance Increase per 1 l Suspension, g/l hr	CO Accumulation in mg per 1 g Dry Substance ($\bar{M} \pm m$)	Number of Experiments
0.04 (0.003÷0.085)	2.07±0.05	7
0.30 (0.170÷0.606)	0.092±0.024	11

The results enable one to advance the assumption that the formation of carbon monoxide is not directly related to the photosynthesis process. By analogy with the formation of endogenous carbon monoxide in the human body (Ref. 16), its formation during the algae cultivation may be related to the oxidizing breakdown of the tetrapyrrole ring of the chlorophyll /8 molecule.

Parallel with this group of studies, Ye. A. Ivanov and I. V. Aleksandrova studied the dynamic and frequency characteristics of an algae suspension, as the object of control (Ref. 3). Employing the low-inertia polarographic method, the authors showed that a jump-like change in the illumination intensity causes a specific negative (reverse) phase in the

photosynthesis reaction in 97% of the cases. This phase lasts 3 minutes on the average, after which the photosynthesis intensity is in accordance with the new level of illumination. The total duration of the transitional process is 8-12 minutes. A subsequent study of the dependence of the transitional process parameters upon the cultivation conditions made it possible for the authors to substantiate an analytical expression for the transmitting functions of the chlorella culture with respect to illumination.

The authors also illustrated that the utilization of the negative phase in the photosynthesis reaction, with a jump-like illumination change, for automatically determining the experimental photosynthesis value, and for deriving the culture from it, makes it possible to reduce the time required for this by 5-6 times (up to 50-60 seconds), as compared with a determination on the basis of the direct reaction period.

The data obtained by the authors indicate the demands made by the chlorella suspension on the high-speed automatic control and regulation system, with respect to the illumination parameter, and justify methods of increasing the speed of the automatic systems.

The complex nature of investigations in highly-productive reactors makes it possible to obtain fairly extensive physiologo-ecological and technological characteristics of algae cultures. These characteristics are requisite both for organizing a highly productive technology and for making preliminary theoretical calculations of the material balance in an algal element when it is modeled in insulated gas-exchange systems and in closed cyclical systems of matter.

The results indicate that the minimum amount of data which are

necessary for any object are no doubt included in an ecological system. One can only speak about the possibility of systematically constructing experimental ecological models, and also about the possibility of subsequently studying the complex intra-system relationships - as the main problem in the experimental ecology of closed systems - when such data are available on each of the proposed links in the system.

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